Original Research Article

In-vitro screening of EMS and Sodium azide induced mutant population at seedling stage for drought tolerance in wheat (*Triticum aestivum* L.)

Abstract

Drought caused by climate change result in water shortages, decreased global wheat production, and unevenly distributed heavy rains. About 50% of the global wheat production is affected by water deficit conditions. The effects of different concentrations of EMS and SA on HD-3226 and HI-1620 wheat genotype for drought tolerance capability were investigated at seedling stage under water and osmotic stress conditions. In the present study, 15% concentration of polyethylene glycol (PEG-6000) was used for in-vitro screening of EMS and SA induced mutant lines of both wheat genotypes. Exposure to PEG-6000 solutions has been effectively used to mimic drought stress. Results of the present study revealed that SA decreased germination of seeds, seedling length, seed vigour index and survival rate percentage while it increased morphological mutation frequency percentage as comparison to EMS according to average mean value of EMS and SA in both wheat genotypes genotype under water and PEG stress condition. Osmotic stress significantly reduced seed germination, shoot length, root length, seed vigour index, survival rate percentage compared to control-wild type.

Keywords

Water stress, seed vigour index, Mutagen, Wheat, Drought stress, Morphological characters

Abbrevaitions

EMS- Ethyl Methane Sulphonate, SA - Sodium Azide, PEG- Polyethylene Glycol

Introduction

Wheat (Triticum aestivum L.) is a member of Triticeae tribe and Poaceae family. Since 10,000 years ago, tetraploid and hexaploid varieties of wheat have been domesticated (Dubcovsky, *et al*., 2007) [1] and Hexaploid form is modern day bread wheat and fulfils dietary needs of global population. By 2050, global population is expected to reach 10

billion, which would require double of current global food production (Mahapara, *et al*., 2022) [2]. Global climate change affects a variety of factors associated with water stress and extreme drought land area is likely to increase from 1to 35% by year 2100 (Kumar, *et al*, 2022)[3]. Bread wheat, being a rabi season crop is exposed to a number of environmental stresses namely, drought, cold, salinity and heat stress among which drought stress is major contributor to crop losses. Moisture or drought stress occurs at all stages irrespective of growth stages which largely depend on local environmental conditions (Manoj, *et al*., 2020) [4]. Drought conditions particularly affect yield and quality of wheat (*Triticum aestivum* L.), one of the most important and widespread grain crops, indispensable in human nutrition and animal feed production (Javadinejad, *et al*., 2021) [5]. Development of stress tolerant varieties is always a main objective of many breeding programs, but success has been limited by adequate screening techniques, and lack of genotypes that show clear differences in response to various environmental stresses (Vukovi´c, *et al*., 2022) [6]. Screening and identification of wheat genotypes that can tolerate water stress is important to boost wheat production which can be achieved by exploring genetic potential from available germplasm of wheat. Seed germination is first stage of growth that is sensitive to water deficit. Therefore, seed germination, vigour and coleoptile length are rudiments for success of stand establishment of crop plants (Khakwani, *et al*., 2011) [7]. Thus there is a need to improve genetic tolerance of crops at seedling stages. Improvement in grain yield of wheat has traditionally relied on direct selection for this trait (Braun *et al*., 1992) [8]. Genetic modification is best method for improving wheat production (Sharma *et al*., 2022)[9]. Induction of mutations by various mutagens is one way to generating large number of variability and has been successfully employed (Kadhimi *et al*., 2016) [10]. Mutant screening involves evaluation of a large number of mutant plants to identify rare mutant individuals that meet desired trait. The screening for drought tolerant lines of wheat under field condition is time consuming and labour intensive. Therefore, there is an urgent need to develop a simple and effective early screening method (Kumar, *et al*., 2018) [11]. For drought stress induction, one of most popular approaches is to use high molecular weight osmotic substances, such as polyethylene glycol (PEG). These agents have no detrimental or toxic effects on plant (Tabatabai *et al* 2022) [12]. They inhibit plant's growth by reducing water potential in a way similar to soil drying (Bressan *et al*., 1981) [13]. It affects how quickly plants' roots and shoots grow and generates osmotic stress in them. (Latif *et al*., 2022) [14]. The non-ionic, almost impermeable chains of PEG molecules (PEG 6000) maintain homogeneity of water potential throughout experiment without harming people's health (Vuković *et al*., 2022)[6].

The research on identifying drought resistant wheat genotypes have demonstrated that various features respond differently with varying amounts of PEG-6000 (Abro *et al*.,2021 and Batool *et al*.,2022) [15,16]. In several studies, it has been observed that PEG based screening may be a option to screen drought tolerant wheat lines at seedling stage. Therefore, present experiment was designed to screening M_1 seeds using PEG solution for drought tolerance in wheat combining with EMS and SA.

Materials and Methods

Mature dry seeds of latest released varieties- HD-3226 (susceptible) and HI- 1620 (tolerance) were used for the present investigation. Seeds (M_0 seeds) of genotypes HD-3226 and HI-1620 were sterilized with 70% ethanol for 3-5 minutes at room temperature and then pre-soaked for 12 hours. Experiment was conducted during Rabi session 2020-2021(M_1 generation). After overnight pre-soaking, one hundred fifty seeds (M_0) of each genotype were treated with four different concentrations of Ethyl Methane sulphonate (EMS)-0.25%, 0.5%, 0.75%, and 1% (v/v) and three different concentrations of sodium azide (SA)-0.02%, 0.04%, and 0.08% (w/v) for 2 hour were used. After mutagen treatment, treated seeds (M_1) seeds were transfer into a small cotton bags and these bags tie on stopcock to remove excess chemical mutagens under running tap water for 2 hours. Then, twenty-five air dried M_1 and non-treated seeds as control (Wild Type) were placed on moistened double whatman filter paper in each Petri plates with three replications in water and 15% PEG-6000 solution (w/v) for drought tolerance screening (Basha and Mehta, 2016) [17]. Five ml of 15% PEG solution was added to each Petri plates under osmotic stress conditions and distilled water was added to each Petri dish under normal conditions every 2 days to compensate for losses through evaporation up to 15 days (El Siddig, *et al*., 2013)[18]. When seedlings were at stage of first true leaf initiation (after 15 days), successful germination in both water and 15% PEG solution, the germination data was collected for calculation of germination percentage, root and shoot length of the germinated seedlings were measured in centimetre before transferring in the small plastic pots and survival data along with morphologically mutant plants, was collected for survival percentage calculation at maturity of M_1 and M_2 generation, respectively. After 20 days, all plants were transferred in the field in a randomly block design (RBD). All treated (M_1) and control seeds (Wild Type) were sown in the field in three replications by maintaining row to row and plant to plant distance. The plant to plant distances was kept around 15 cm and spacing between adjacent rows was kept 20cm. The gap between two rows of different genotype was kept 50cm for maintaining proper distance from each genotype to

another genotype. After transplanting of all plants, fields were irrigated at regular interval of 20-25 days. The crop was maintained in the field using conventional agronomic practices to keep crop in good condition. All plants (mutant as well as control- Wild Type) sown in field, were tagged properly with their genotype name along with number and other details. For drought screening at seedling stage data were calculated using following formulas:

1. Germination %

Germination % is an estimate of viability of a population of seeds. The germination % of seeds was measured by counting total germinated seeds from the total seeds were sown for the experiment after 15 days of sowing.

$$\textbf{Germination percentage (GP)} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

2. Seedling Length (SLs)

The length of seedlings was measured in centimetre from starting point of root tip to the top node of shoot using a ruler against a millimetre paper.

3. Seed Vigour Index [SI (%)]

The vigour index of each seed (control-WT and treated seed) was calculated using the formula which proposed by Abdul-Baki and Anderson (1973) [19] as germination percentage multiplied by seedling length.

$$\textbf{Seed Vigour Index (SI)} = \text{Germination percentage} \times \text{Seedling Length}$$

Where,

Seedling Length = Root length + Shoot length in cm,

4. Survival Rate % [SR]

The surviving plants in control and different treatments were counted at the time of maturity and calculated by following formula:

$$\textbf{Survival rate \%} = \frac{\text{Number of living seedlings}}{\text{Number of Total seedlings}} \times 100$$

5. Morphological Mutation Frequency [MF%]

Morphological mutations were screened throughout the growth period of plants and Gustafsson method (1940)[20] used for mutation frequency calculation.

$$\textbf{Morphological Mutation Frequency} = \frac{\text{Number of mutated plants}}{} \times 100$$

Number of Total plants

Statistical Analysis

The recorded data were subjected to analysis of variance based on Random Block Design (RBD) with two factors was performed by OPSTAT software.

Result and Discussion

The in vitro screening of M_1 populations was carried out by germination of seeds on 15% PEG-6000 and analysing the mutagenic effects of EMS and Sodium azide were studied on seed germination, seedling length, seed vigour index, plant survival in M_1 generation while morphological mutation frequency in M_2 generation of both wheat genotype.

Germination %

In HD-3226 genotype, seed germination in control-wild type was 97.00% in water and 96.67% in 15% PEG (Table1). In case of EMS, it range from 94.67% (EMS 0.75%) to 98.33% (EMS 0.5%) and 88.67% (EMS 0.5%) to 94.00% (EMS 0.75%) in water and 15% PEG treatments, respectively. While SA decreased the germination of seeds as comparison to EMS, it range from 84.67% (SA 0.04%) to 89.33% (SA 0.02%) and 88.00% (SA 0.04%) to 90.33% (SA 0.08%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 97.08% (EMS) and 86.33% (SA) while it was 90.41% (EMS) and 89.22% (SA) in PEG. In HI-1620 genotype, seed germination in control-wild type was 97.33% in water and 98.67% in 15% PEG. In case of EMS, it range from 97.33% (EMS 0.75%) to 98.67% (EMS 0.25%, EMS 0.5% and EMS 1%) and 88.00% (EMS 1%) to 93.33% (EMS 0.5%) in water and 15% PEG, respectively. SA decreased the germination of seeds as comparison to EMS in water and it range from 88.00% (SA 0.04% and SA 0.08%) to 92.00% (SA 0.02%) while SA increased the germination of seeds as comparison to EMS in PEG and it range from and 90.67% (SA 0.02% and SA 0.08%) to 96.00% (SA 0.04%). The average mean value across all treatments in water was 98.33% (EMS) and 89.33% (SA) while it was 90.66% (EMS) and 92.44% (SA) in PEG. The germination percentage decreased in PEG as comparison to water in EMS treatments while it increased in PEG as comparison to water in SA treatments according to average mean value of EMS and SA in both wheat genotypes (Table1). In mutagenic treatments reduction in seed germination is due to delay or inhibition in physiological and biological processes necessary for seed germination which includes

inhibition of mitotic process (Sato and Gaul, 1967) [21], enzyme activity (Chrispeeds and Varner, 1967) [22] and hormonal imbalance (Ananthaswamy *et al*., 1971) [23]. Similar findings have also been reported, like reduction in germination rate with the increase PEG were noted in different crops (Basha *et al*., 2015; Kadhimi *et al*., 2016 and Sharma, *et al*., 2022)[17,10,9]. Rajoriya *et al*., 2016, also reported that seed germination decreased with increase in concentration of mutagens [24].

Table 1: Germination percentage of seeds of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate (EMS) and Sodium azide (SA) treatments in water and 15% PEG for *in-vitro* screening at seedling stage

Treatment	HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean
Control	97.00	96.67	96.83	97.33	98.67	98.00
EMS 0.25%	97.33	89.33	93.33	98.67	90.67	94.67
EMS 0.5%	98.33	88.67	93.50	98.67	93.33	96.00
EMS 0.75%	94.67	94.00	94.33	97.33	90.67	94.00
EMS 1%	98.00	89.67	93.83	98.67	88.00	93.33
SA 0.02%	89.33	89.33	89.33	92.00	90.67	91.33
SA 0.04%	84.67	88.00	86.33	88.00	96.00	92.00
SA 0.08%	85.00	90.33	87.67	88.00	90.67	89.33
EMS Mean	97.08	90.41	93.74	98.33	90.66	94.49
SA Mean	86.33	89.22	87.77	89.33	92.44	90.88
Total Mean	93.04	90.75		94.83	92.33	
	Factor (A)	Factor (B)	Factor (A×B)	Factor (A)	Factor (B)	Factor (A×B)
C.D.	3.87	1.94	5.48	3.52	1.76	4.98
SE(d)	1.89	0.94	2.67	1.72	0.86	2.43
SE(m)	1.33	0.67	1.89	1.21	0.61	1.72

Seedling Length (cm)

In HD-3226 genotype, seedling length in control-wild type was 33.90cm in water and 15.97cm in 15% PEG (Table 2). In case of EMS, it ranges from 22.81cm (EMS 0.5%) to 29.95cm (EMS 0.25%) and 16.62cm (EMS 1%) to 22.68% (EMS 0.75%) in water and 15% PEG treatments, respectively. While SA decreased the seedling length as comparison to EMS, it range from 15.73cm (SA 0.04%) to 22.23cm (SA 0.08%) and 11.91cm (SA 0.08%) to 17.74cm (SA 0.04%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 26.84cm (EMS) and 18.87cm (SA)

while it was 20.38cm (EMS) and 15.19cm (SA) in PEG. In HI-1620 genotype, seedling length in control-wild type was 19.73cm in water and 19.12cm in 15% PEG. In case of EMS, it ranges from 23.78cm (EMS 1%) to 28.99cm (EMS 0.5%) and 15.96cm (EMS 1%) to 19.17cm (EMS 0.75%) in water and 15% PEG, respectively. SA also decreased seedling length as comparison to EMS, it range from 18.50cm (SA 0.08%) to 19.07cm (SA 0.02%) and 15.27cm (SA 0.04%) to 15.73cm (SA 0.02%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 26.22cm (EMS) and 18.87cm (SA) while it was 17.67cm (EMS) and 15.53cm (SA) in PEG. Seedling length decreased in PEG as comparison to water in both EMS and SA treatments according to average mean value of EMS and SA in both wheat genotypes. Roots are important in up taking water and nutrients, perceiving and transducing water deficit to shoots which will trigger different morpho-physiological responses and it was an important trait for selection of drought resistant genotypes. According to Fraser *et al*., (1990) [25], reduction in root and shoot lengths may be due to an impediment of cell division and elongation leading to a kind of tuberization which allow the stressed conditions to become favourable. Similarly, Rajoriya *et al*., 2016 [24] reported that seedling length decreased with increase in concentration of mutagens. Similar results also reported by Vukovi´c *et al*., 2022 and Memon *et al*., 2023[26], seedling length of wheat cultivars significantly decreased under osmotic stress.

Table 2: Seedling length (cm) of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate (EMS) and Sodium azide (SA) treatments in water and 15% PEG for *in-vitro* screening at seedling stage

Treatment	HD 3226			HI 1620		
	Water	PEG	Mean	Water	PEG	Mean
Control	33.90	15.97	24.93	19.73	19.12	19.47
EMS 0.25%	29.95	21.49	25.69	28.35	17.67	23.01
EMS 0.5%	22.81	20.76	21.78	28.99	17.91	23.47
EMS 0.75%	28.87	22.68	25.77	23.79	19.17	21.48
EMS 1%	25.74	16.62	21.18	23.78	15.96	19.87
SA 0.02%	18.65	15.93	17.29	19.07	15.73	17.40
SA 0.04%	15.73	17.74	16.74	19.05	15.27	17.16
SA 0.08%	22.23	11.91	17.076	18.50	15.60	17.05
EMS Mean	26.84	20.38	23.61	26.22	17.67	21.94
SA Mean	18.87	15.19	17.03	18.87	15.53	17.20
Total Mean	24.73	17.88		22.66	17.05	
	Factor (A)	Factor (B)	Factor (A×B)	Factor (A)	Factor (B)	Factor (A×B)

C.D.	1.19	0.59	1.68	1.93	0.97	2.75
SE(d)	0.59	0.29	0.82	0.94	0.47	1.33
SE(m)	0.41	0.20	0.59	0.66	0.33	0.94

Seed Vigour Index [SI (%)]

In HD-3226 genotype, seed vigour index in control-wild type was 32.86% in water and 15.44% in 15% PEG (Table 3). In case of EMS, it range from 22.43% (EMS 0.5%) to 29.13% (EMS 0.25%) and 14.92% (EMS 1%) to 21.32% (EMS 0.75%) in water and 15% PEG treatments, respectively. While SA decreased the seed vigour index as comparison to EMS, it range from 13.31% (SA 0.04%) to 18.89% (SA 0.08%) and 10.74% (SA 0.08%) to 15.63% (SA 0.04%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 26.03% (EMS) and 16.29% (SA) while it was 18.45% (EMS) and 13.54% (SA) in PEG. In HI-1620 genotype, seed vigour index in control-wild type was 19.20% in water and 18.87% in 15% PEG. In case of EMS, it range from 23.16% (EMS 0.75%) to 28.61% (EMS 0.5%) and 14.02% (EMS 1%) to 17.39% (EMS 0.75%) in water and 15% PEG, respectively. SA decreased the seed vigour index as comparison to EMS, it range from 16.23% (SA 0.08%) to 17.50% (SA 0.02%) and 14.15% (SA 0.08%) to 14.67% (SA 0.04%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 25.80% (EMS) and 16.84% (SA) while it was 16.04% (EMS) and 14.37% (SA) in PEG. The seed vigour index decreased in PEG as comparison to water in both EMS and SA treatments according to average mean value of EMS and SA in both wheat genotypes. Similar findings also reported by Arisandy *et al*. (2017) [27] and Nirmal-Raj *et al*. (2019) [28] in maize, seed vigour indices reduced under PEG induced drought.

Table 3: Seed Vigour Index of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate (EMS) and Sodium azide (SA) treatments in water and 15% PEG for *in-vitro* screening at seedling stage

Treatment	HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean
Control	32.86	15.44	24.15	19.20	18.87	19.03
EMS 0.25%	29.13	19.17	24.15	27.97	16.03	22.00
EMS 0.5%	22.43	18.41	20.42	28.61	16.75	22.68
EMS 0.75%	27.35	21.32	24.33	23.16	17.39	20.27
EMS 1%	25.24	14.92	20.08	23.47	14.02	18.75
SA 0.02%	16.69	14.25	15.47	17.50	14.29	15.90
SA 0.04%	13.31	15.63	14.47	16.81	14.67	15.74

SA 0.08%	18.89	10.74	14.81	16.23	14.15	15.19
EMS Mean	26.03	18.45	22.24	25.80	16.04	20.92
SA Mean	16.29	13.54	14.91	16.84	14.37	15.60
Total Mean	23.24	16.23		21.62	15.77	
	Factor (A)	Factor (B)	Factor (A×B)	Factor (A)	Factor (B)	Factor (A×B)
C.D.	1.43	0.72	2.02	1.92	0.96	2.72
SE(d)	0.70	0.35	0.99	0.94	0.47	1.33
SE(m)	0.49	0.25	0.70	0.66	0.33	0.94

Survival Rate % [SR]

In HD-3226 genotype, survival rate percentage in control-wild type was 99.33% in water and 99.00% in 15% PEG (Table 4). In case of EMS, it range from 87.67% (EMS 0.75%) to 98.52% (EMS 0.5%) and 89.06% (EMS 1%) to 93.17% (EMS 0.75%) in water and 15% PEG treatments, respectively. While SA decreased survival rate percentage as comparison to EMS, it range from 86.28% (SA 0.02%) to 87.78% (SA 0.08%) and 86.39% (SA 0.04%) to 87.78% (SA 0.02%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 95.01% (EMS) and 87.23% (SA) while it was 91.01% (EMS) and 87.28% (SA) in PEG. In HI-1620 genotype, survival rate percentage in control-wild type was 98.67% in water and 98.01% in 15% PEG. In case of EMS, it range from 89.05% (EMS 0.75%) to 96.89% (EMS 1%) and 87.03% (EMS 0.75%) to 91.17% (EMS 0.25%) in water and 15% PEG, respectively. SA decreased survival rate percentage in compare to EMS, it range from 90.11% (SA 0.04%) to 90.39% (SA 0.02% and SA 0.08%) and 88.13% (SA 0.02%) to 91.44% (SA 0.08%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 92.04% (EMS) and 90.29% (SA) while it was 89.22% (EMS) and 89.56% (SA) in PEG. The survival rate percentage decreased in PEG as comparison to water in both EMS and SA treatments according to average mean value of EMS and SA in both wheat genotypes. According to Natrajan and Shivshankar (1965) [29] and Sato and Gaul (1967) [21], reduction in seedling survival can be attributed to cytogenetic damage and physiological disturbances caused by mutagen treatment thus, seedling survivability may be hindrance caused by EMS and SA on different metabolic pathways of cells. Similar findings also reported by Rachovska and Dimova (2000) [30] in wheat, Khan *et al*. (2004) [31] in mungbean, Ilbas *et al*. (2005)[32] in barley and Kumar and Srivastava, (2013)[33] in Sesbania. Rajoriya *et al*. (2016) [24] also reported that plant servival decreased with increase in concentration of mutagens.

Table 4: Survival rate percentage of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate (EMS) and Sodium azide (SA) treatments in water and 15% PEG for *in-vitro* screening at seedling stage

Treatment	HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean
Control	99.33	99.00	99.16	98.67	98.01	98.33
EMS 0.25%	97.97	91.45	94.69	90.44	91.17	90.80
EMS 0.5%	98.52	90.39	94.45	91.78	88.33	90.07
EMS 0.75%	87.67	93.17	90.42	89.05	87.03	88.03
EMS 1%	95.89	89.06	92.47	96.89	90.38	93.67
SA 0.02%	86.28	87.78	87.03	90.39	88.13	89.22
SA 0.04%	87.63	86.39	87.02	90.11	89.11	89.62
SA 0.08%	87.78	87.67	87.73	90.39	91.44	90.91
EMS Mean	95.01	91.01	93.01	92.04	89.22	90.63
SA Mean	87.23	87.28	87.25	90.29	89.56	89.92
Total Mean	92.69	90.63		92.27	90.45	
	Factor (A)	Factor (B)	Factor (A×B)	Factor (A)	Factor (B)	Factor (A×B)
C.D.	3.94	1.96	5.59	2.77	1.36	N/A
SE(d)	1.92	0.95	2.74	1.39	0.64	1.89
SE(m)	1.35	0.67	1.92	0.94	0.47	1.39

Morphological Mutation Frequency [MF%]

In present study, several morphological mutants showing variations in characters like altered plant height, leaf architecture, growth habit, tillers per plant, spike length and morphology etc. were isolated in M_2 generation of wheat genotypes. In HD-3226 genotype, morphological mutation frequency of EMS range from 3.38% (EMS 0.5%) to 4.70% (EMS 0.75%) in water and 3.44% (EMS 0.25%) to 5.33% (EMS 0.75%) in water and 15% PEG treatments, respectively (Table 5). While SA increased morphological mutation frequency as comparison to EMS, it range from 3.63% (SA 0.04%) to 4.84% (SA 0.08%) and 3.66% (SA 0.08%) to 6.28% (SA 0.04%) in water and 15% PEG treatments, respectively. The average mean value across all treatments in water was 4.14% (EMS) and 4.42% (SA) while it was 4.20% (EMS) and 4.88% (SA) in PEG. In HI-1620 genotype, morphological mutation frequency of EMS range from 2.81% (EMS 0.25%) to 4.68% (EMS 0.75%) in water and 3.04% (EMS 0.5%) to 4.92% (EMS1%) in water and 15% PEG treatments, respectively. While SA increased morphological mutation frequency as comparison to EMS, it range from 3.39% (SA 0.02%) to 4.56% (SA 0.08%) and 4.03% (SA 0.04%) to 5.95% (SA 0.02%) in water and 15% PEG treatments, respectively. The average mean

value across all treatments in water was 4.05% (EMS) and 4.17% (SA) while it was 4.13% (EMS) and 4.84% (SA) in PEG. The morphological mutation frequency increased in PEG as comparison to water in both EMS and SA treatments according to average mean value of EMS and SA in both wheat genotypes. Tokar (2009)[34] reported that morphological mutants are useful in gene mapping and phylogenetic studies of crops. Khursheed *et al*., (2019)[35] reported highest morphological mutation frequency in gamma rays and lowest with combined treatments in faba beans. Similarly, Raina and Khan, (2020) [36] also reported the highest mutation frequency with combination of gamma rays and sodium azide (5.86%) in cowpea.

Table 5: Morphological mutation frequency of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate (EMS) and Sodium azide (SA) treatments in water and 15% PEG for *in-vitro* screening at seedling stage

Treatment	HD- 3226			HI- 1620		
	water	PEG	Mean	water	PEG	Mean
EMS 0.25%	4.32	3.44	3.88	2.81	4.56	3.68
EMS 0.5%	3.38	3.47	3.42	4.14	3.04	3.59
EMS 0.75%	4.70	5.53	5.11	4.68	4.03	4.36
EMS- 1%	4.18	4.65	4.41	4.57	4.92	4.74
SA- 0.02%	4.79	4.69	4.74	3.39	5.95	4.67
SA- 0.04%	3.63	6.28	4.95	4.54	4.03	4.29
SA- 0.08%	4.84	3.66	4.25	4.56	4.54	4.55
EMS Mean	4.14	4.2	4.17	4.05	4.13	4.09
SA Mean	4.42	4.88	4.68	4.17	4.84	4.50
Total Mean	4.266	4.535		4.105	4.442	
C.D.	1.026	N/A	1.45	0.832	N/A	1.176
SE(d)	0.496	0.265	0.702	0.402	0.215	0.569
SE(m)	0.351	0.188	0.496	0.285	0.152	0.402

Conclusion

Water is essential for seed germination, seedling growth, vegetative period of crop, flowering at translocation of minerals and nutrition incorporate throughout the plants, from root to leaf and vice versa in the plants (Kijne, 2006) [37]. Water stress is a major limiting factor for crop production and estimated the 50% of the global wheat production is affected by water deficit conditions (Wang *et al*., 2003) [38]. Crops produced in countries with frequent drought can become more drought-resistant through mutation breeding. In vitro screening method using PEG has been proved to be very effective method for studying the effect of water stress on seed germination and seedling growth characters (Hadas, 1976; Aquila *et al*., 1984; Van den

Berg & Zeng, 2006 and Radhouane, 2007) [39, 40, 41]and simple cost effective method to screen large set of germplasm within very less time period and accurately (Kulkarni, 2007) [42]. However, artificial induction of drought using PEG is dependent on concentration and varies with crop and genotype. In present investigation, osmotic stress significantly reduced seed germination, shoot length, root length, seed vigour index, survival rate percentage compared to the control-wild type. SA decreased germination of seeds, seedling length, seed vigour index and survival rate percentage while it increased morphological mutation frequency percentage as comparison to EMS according to average mean value of EMS and SA in both wheat genotypes genotype under water and 15% PEG stress condition. Considering the present data, variety HD-3226 was drought susceptible, while variety HI-1620 showed greater drought stress tolerance, showing better germination under osmotic stress.

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